The Anatomy and Morphology of Simulium Vittatum

by H.B. Hungerford

1913

Submitted to the Department of Entomology of the University of Kansas, in partial fulfillment of the requirements for the Degree of Master of Arts

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A THESIS

SUBMITTED TO THE DEPARTMENT OF ENTOMOLOGY OF THE UNIVERSITY OF KANSAS, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS

BY

H. B. HUNGERFORD UNIVERSITY OF KANSAS.

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OUTLINE OF THESIS.

Introduction.

Taxonomic Position and Description of Form Studied.

General Habits and Life History of the Family.

Technique used in the Study of Gross Anatomy.

External anatomy : Head, Thorax, Abdomen.

Internal Anatomy:

- The Skeletal Invaginations.
- The Muscular System.
- The Circulatory System.
- The Digestive System.
- The Respiratory System.
- The Reproductive System.

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ACKNOWLEDGMENTS.

The preparation of this paper was undertaken in connection with the experimental work upon the Sandfly and Pellegra, being carried on by Professor Hunter of the University of Kansas.

The anatomical research was taken up at the suggestion of Professor Hunter, and the writer desires herewith to express his appreciation to him for his kindly interest and helpful suggestions during the progress of the work.

He also wishes to acknowledge the aid given him by Mr. F. X. Williams in reviewing and correcting the manuscript, by Miss Ruby Hosford in loaning material for sectional study, and by Mr. W. F. Emery in collecting and preserving material for the work.

" ANATOMY OF SIMULIUM VITTUM."

INTRODUCTION.

The recent widespread interest in the sandfly as the possible carrier of the disease "Pellegra" has given the writer a desire to know something of the anatomy and histology of the form common in Kansas, namely Simulium vittatum.

An anatomical study of this form has been found necessary in order that proper orientation might be made in the searching examinations given for protozoa and their other parasitic forms within the bodies of these insects.

The study of the gross structures of Simulium has been of some scientific interest aside from its possible connection with Pellegra, for so far as the writer has been able to find, no morphological study of the adult has been made aside from one paper by F. H. Taylor, 1902, on the "Tracheal System"of Simulium", and studies of the mouth structures by Smith, Meinert and Maccloskie.

A number of writers have studied the larvae and pupae in connection with some general problem dealing with a group of related forms. Thus Osten Sachen '70 made a study of the transformations of Simulium species, and Packard '72 in his "Embryological Studies on Hexopodous Insects" studied the development of Simulium. Kellggg and Vaney (1901) used the Simulium along with Cheronomids and like forms in their study of "Phogocytosis in the Postembryonic Development of Diptera". Vaney (1902) continueing his studies of the Fat Bodies examined Sandflies and other inferior diptera as he calls them (Culex, Chironimids, etc). Weismann in studying imaginal buds referred to S. Sericea and Miall 1900 in his researches on the respiratory appendages of pupae figures a Simulium sp. Headlee '06 made a study of the blood gills of the larva of S. pictipes, and Dutt in an unpublished article at Cornell recorded his work upon the silk glands of Simulium larvae. So scattered and fragmentary has been the morphological work upon this important insect pest that it is the desire of the writer to make an exhaustive study of this form. The present paper deals with the

external and internal anatomy of the dominant Kansas species and gives special attention to those anatomical parts that might harbor parasitic forms, namely the digestive canal and salivary glands.

TAXONOMIC POSITION AND DESCRIPTION OF FORM STUDIED.

Simulium vittum is a small compactly built fly belonging to the family "Simulidae"

The Simulidae are known in the vernacular as "Turkey gnats", "Blackfly," and "Sandflies." They are related in a general way to the mosquitos craneflies, fungus gnats, and punkies, but may be readily distinguished from them by their general stoutness of body and broadness of wing. The legs of the mosquitos and of most gnats are long and rather slender, but those of the sandflies are short and stout. Their general form is so characteristic that they are easily recoginzed. They possess very brodd wings. Their bodies are short and the thorax so much arched that they appear as Johannsen espresses it "humpbacked" (see Fig.1 Plate I)

Technically the adults of this family are distinguished by the following characteristics; -

Antennae but little larger than the broad, flattened or cylindrical 10 jointed. The two basal joints differentiated, the others closely united and never plumose.

The Eyes round or reniform and holoptic in the male - ocelli absent.

Proboscis not elongated and possessing small horny labella and 4-jointed palps. The first

joint of the palp is short - the two following of equal length, and the last one longer and more slender than the preceding.

The Thorax is arched, without a suture, and the scutellum small.

The Abdomen is cylindrical, made up of 7 or 8, (or more) segments and the genitalia concealed.

The Legs are strong and not elongate, the femora broad and flat, tibia usually with terminal spurs; 1st joint of tarsi longer than the following and usually dilated in the male, the last joint small; Wings large and broad, with distinct alulae, anterior veins thickened, the others slender, auxiliary vein terminating in the costa about the middle of the wing which is not continuous beyond the tip of the wing. (see Fig. - 1 Pl), second longitudinal vein wanting, the first and third lying close to each other, the third arising from the first rectangularly before the end of the auxiliary vein; anterior cross vein very short, fourth vein curved, forked nearly opposite the anterior cross vein, the forks terminating near the tip of the wing. (see Fig. I Pl I)

Simimium vittatum - Zetterstedt

(tribulatum Luggar, decorum (walk), argus (will)

Adult.

Female

"Gray - nearly bare; dorsum of thorax with five black stripes, the median one entire, the intermediate pairs interrupted - the exterior pair spotlike. Each segment of the abdomen with a black dorsal stripe, and basally on each side with a black spot, the penultimate segment black.

Wings whitish hyoline; halteres white; legs fuscous black, the front side of the anterior tibiae, the base of the middle and hind tibiae, and the base of the middle and hind metatarsi white., length 3 mm".

(Zetterstedt)

Loquelett adds the following : " Female :- abdomen gray, bases of segments 3 to 7 or 8 marked with a velvet black fascia produced backward in the middle and at the ends, length 2 to 4 mm. Found in N.Y., Minn., Kansas, Illinois, and Cal."

Male - Hind tarsi bicolorous, mesonotum gray on sides and hind margin - center largely velvet black; without gray streak extending inward from humerous, sides of abdominal segemts 4 to 7 with silvery white hairs - (coquillett).To this Johannsen adds :-

"The markings of the female of this species seem somewhat variable, the thoracic markings are usually quite distinct, the median stripe is nearly of uniform width excepting at the posterior end, where it becomes narrower; the intermediate stripes are ∫ shaped, the extremities larger, the intermediate portion usually a hair line, sometimes obsolete, the exterior pair usually elongated spots. The abdominal markings are as described by Coquillet, though occasionally there are additional disconnected, velvet black lateral spots, one on each side on segments 3,4, and 7, and a pair on 5 and 6. Sometimes also, owing either to the contracted condition of the abdomen or to the fasciae being narrow, only the black projections of the fasciae are visible on the more posterior segments, giving the appearance of three spots on each. The legs are often gray, the femora and tibiae paler at the base, the tibiae black at tip, the tarsi deep black except basal portion of middle and hind metatarsi, fore tibiae with one spur, middle and hind with one pair. Tarsal claws of female simple.

LARVAE.

Caudal blood gills, 3 simple papillae; the middle tooth of the labium simple and pointed, labium with six pairs of setae on its ventral surface; somewhat mottled gray, the sides of each segment blackish;

The head is of the usual reddish brown color, the pale yellow antennae long and cylindric, the second joint about one-third of the length of the first, the third is a pointed process at the tip of the second. The fans have about 40 rays, the cilia being relatively minute; the mandibles are provided with three large apical teeth besides the row of secondary ones; the apical pair of bristles is present; the maxillary palpus has a few spines, and a tuft of a few spines on the basal joint; hypopharynx and labrum apparently like those of other species; the labrum has an elongate middle tooth, those at the end nearly as long, the intermediate ones short, and there are six bristles in each of the two longitudinal rows on the ventral surface; the three blood gills at caudal end unbranched.

PUPA

The thoracic respiratory filaments each consist of a single main trunk, from which arise eight branches each of which divided into two, thus making 16 twigs in all (Pl fig.) Near the basal margin of the last few abdominal segments are a few caudal projecting dorsal hooks, and on the tip of the last segment is a pair of blunt spines. The pupal case is of the wall-pocket type, from which the respiratory filaments of the pupa project.

GENERAL HABITS AND LIFE HISTORY OF THE FAMILY.

The habits of the adults are quite generally known because in many places they are fully as troublesome as mosquitos. They are so called "bloodsuckers" feeding upon the blood of horses, cattle, and man, sometimes to such as extent as to be of considerable economic importance- (see Riley in N.Y. State Mus. Bull 68) They lay their eggs on the surface of rocks over which shallow water is flowing rapidly, as on the rocks of shallow rapids and the ledges of waterfalls. The eggs are laid in masses one layer deep. In about a week they hatch into larvae which attach themselves by their caudal end to the rocks or stems of aquatic plants.

They retain their position in the swiftest current by means of a disc-like sucker on the posterior end of the body. When they desire to move to a new location they may do so by anchoring a silken thread to the support and, releasing their hold, spinning out their silk as they are washed down stream, but retaining control of it very much after the fashion of spiders, suspending themselves in mid-air, or they may travel over the surface of the rocks with a looping gait similar to that of a measuring worm.

They are said to feed upon algae, diatoms, and parts of phaneragamous plants* On those I have examined diatoms have comprised most of the material found in the digestive canal.

When the larva is ready to transform it spins a tough pupal case firmly attached to the rock and changes to a strangely shaped pupa. Then in due time the fly comes from the pupal skin, rises to the surface of the water and takes flight at once. Soon there occurs the mating and very shortly thereafter the eggs are laid for the next generation, and the life cycle of the sandfly is repeated.

* Miss R. Phillips 1890 of Cornell University reports that among the algae taken by the larvae are Nothix, Chidophora, and Vaucherio.

TECHNIQUE FOR THE STUDY OF GROSS ANATOMY.

Most of the studies of the gross anatomy of the Simulium fly were made by means of free dissections under the binocular.* These studies were verified by serial microtome sections.

The comparatively small size of the fly (3 mm) necessitated the use of a technique somewhat more delicate than is usually necessary, and in as much as the majority of students of small forms have failed to record their methods of study, I have felt it desirable to give a brief account of the technique used in these studies.

The needles used in the dissection work were made by placing the base of minuten nadeln points (used in mounting small insects) in handles of wood and grinding the points on emery paper until they were small enough for use.

The flies were held in any given position by paraffin. To do this, some paraffin was placed in a small shallow porcelain straining dish, and warmed to melting point. The fly was then placed about half its width in the paraffin in the desired position and the paraffin cooled.

*I used a Zeiss machine and found that the No.2 eye piece used with the A_2 objective gave by far the most satisfactory results for the study of general anatomy. Quite a number of the body tissues of the fly are trans-

parent, and for a study of these the direct sunlight gave the best illumination.

**

The dish was then filled with either normal salt solution or with 50% alcohol depending upon whether the material was fresh or fixed. With careful work and a steady hand one could determine the various systems with considerable accuracy. One fly would usually serve for several days dissection study, and by covering the dish with a lid, and inverting a tumbler over all to prevent evaporation of the aclohol the specimen would be ready for further study without the usual delay.

Most of the dissections were made with the fly on its side. The large muscles of the thorax and the deep incision of the exoskeleton between the thorax and the abdomen made dorsal dissections quite unsatisfactory. Ventral dissections were used only to verify the other studied by giving a different point of view.

Sectional Study.

Killing and Fixing.

In order to determine the best method of fixing and staining the adult flies it was necessary to try a number of fixations and stains.

The flies were divided into lots and killed and fixed in the following ways :

In lots I to III live sandflies were placed into Picro-aceto-sublimate for 12, 18, and 24 hours respectively. When they had been in the fluid the specified length of time, the liquid was carefully drawn off with a pipette, and the vials filled with 70% grain alcohol. At the end of 24 hours the alcohol was changed by the pipette method as above , and this process of changing the alcohol repeated for 3 days. At the end of that time the 70% was replaced with 85% alcohol and stored.

In lots IV to VI the sandflies were taken from the arm cage of the pellegra patient and fixed as described above.

In lots VII, VIII, IX live sandflies were captured and placed in Picro-formal for 12 hours, 18 hours, and until they sank respectively. They were then washed out with 70% alcohol as for the Picro-aceto-Sublimate and stored in 85% alcohol.

In lots X, XI, XII the live flies were removed from the arm cage and fixed in Picro-formal as described above.

Lot XIII was fixed in Flemings' stronger mixture. The flies were allowed to remain in the fixing fluid until they sank. Then they were changed to water and washed for three days. From the water they were transferred to 95% alcohol.

In lot XIV flies were placed from the arm cage

into the Flemings' and left till they sank, after which they were treated as in lot XIII.

In lots XV, XVI, XVII live sand flies were fixed in Gilson's Chloro-aceto-sublimate for 10 minutes, 20 minutes, and until they sank. They were then washed in 70% alcohol for several days and stored in 85 % alcohol.

In lots XVIII, XIX, XX flies were taken from the arm cage and treated as above.

In lots XXI, XXII, and XXIII unexposed flies were fixed in Zenker's fluid for 6 hours, 12 hours, and until they sank respectively. They were then washed out in water and transferred to alcohol containing tincture of iodine.

In lots XXIV, XXV, XXVI the flies were taken from arm cage and treated as above.

In the above series the Chloro-aceto-sublimate gave the best fixation.

C. G. Hewett '07 in his work with the housefly used the following: Henning's solution made up as follows: Nitric Acid 16 parts, chromic acid (.5%) 16 parts, picric acid saturated in water 12 parts, and absolute alcohol 42 parts. After fixing the material he washed out in iodine water. This method he finds not only fixes, but to a certain extent softens the chitin, which facilitates sectioning, providing the material is not allowed to be imbedded too long.

Hot alcohol and hot Gilson's both give very good results as has been noted by Headley '06 in his works on Simulium blood Gills.

Specimens killed in hot PerenyesFluid, cut and removed to fresh fluid for six hours and then transferred to 70% alcohol were well fixed.

But the best fixation that I have tried was obtained by the use of Kables Fixation. This was recommended to me by Dr. Brues of Harvard who used it as a fixation for the Stomoxys calcitrans that had been allowed to bite patients having Infantile paralysis. It is made up as follows: 30 parts water, 15 parts normal 95% alcohol, 6 parts Formaline (40%), 1 part of Glacial acetic acid. The flies are killed in this hot solution and transferred when cold to 70% alcohol.

Since the Gilson's mixture mentioned above gave very satisfactory results it might also be well to give directions for making it. To make up a two liter bottle full use :-

30 cc of 80% nitric acid
8 cc of glacial aciter acid.
40 grs Corrosive Sublimate
200 cc of 60% alcohol
1760 cc of distilled water.

INFILTRATING AND INBEDDING.

When the specimens were desired for study, they were graded up from the 85% alcohol to absolute alcohol in small glass dishes covered with ground glass covers. If they were stored in 85% alcohol they were stepped up to 95% and left for 48 hours to harden. Then from this they were transferred to absolute and left for a like time. From this they were transferred to a vial which had been half filled with xylol and then filled with absolute alcohol. Here they were left for 24 hours after which they were returned to a glass dish of pure xylol. The dish was then placed on the top shelf of an electric drying oven, and a few flakes of paraffin dropped into it. When these dissolved more were added and the dish placed nearer the heat. Bits of paraffin were added from time to time for the following six hours. By the end of that time the flies could be transferred to pure melted paraffin and left there for 24 hours or longer.

CUTTING AND MOUNTING.

When the flies were infiltrated they were imbedded and cut in serial sections of from 4 to 15 microns in thickness.

The dry paraffin ribbons were placed on an absolutely clean slide and floated with prepared egg albumen. The albumen was then drained off and the slide held just enough below the surface of tepid distilled water to float out the folds and allow the ribbons to be arranged. The slides were then air dried for one week, after which they were warmed enough to melt down the paraffin and placed in xylol for ten minutes. From the xybol they were passed down thru the alcohols to the grade used in the stain.

STAINING.

Five stains were used in the studies made with the Simulium fly.

I. For thin sections to be studied for parasitic forms Gemsa's Lasung was used. This stain was diluted 1 drop to 1 cc of water made faintly alkaline with weak potassium carbonate (1%). The sections were stained with this for 15 minutes . (With this stain chomatin is stained blue and protozoa and bacteria pinkish) Differentiation was made with tap water. After dehydrating in the alcohols they were transferred to xylol and from the xylol to the balsam mount.

II. For ordinary work the sections of any thickness were run down to 50% alcohol and stained for 12 hours in Borax Carmine, differentiated in acid alcohol, dehydrated and mounted as above.

III. Iron haematoxylin was used in the usual way.

IV. Good results were obtained by overstaining with Delafield's Hämatoxylin and differentiating with acid alcohol as suggested by Hewett..

V. The best results were obtained by running the sections down to 25% alcohol and staining in Ferric Alum for 4 hours, then in Haedenhain's Haematoxylin (5% aq. solution) for 12 hours. Following this thwy were bleached in Ferric alum, placed in Orange G. and dipped back and forth in the two stains till

proper results were obtained as determined by following the action of the stains under the low power compound. Then they were dehydrated, passed up thru Xylol and mounted.

THE THORAX OF SIMILIUM.

The thorax of Dipterous insects always presents a problem. The fact that they possess but one pair of wings has led to the adjusting of the thoracic box to meet this condition. Thus the mesothoracic dividion which bears the wings is greatly developed while the prothoracic and metathoracic divisions are reduced. Time has not permitted a detailed report on the homologies of the several sclerites making up the prothorax. Suffice it to say that the prothoracic cylinder is not typical. The sclerites conforming not at all to our conception of the normal arrangement of parts. The mesothorax, on the other hand, is a simpler matter. The notum is large, occupying the whole of the dorsal side of the thorax. It is not transversed by well marked sutures that could mark the preseutum, scutum, scutullem and postscutellum. As a whole it is a convex shield. It is this sclerite that gives to Simulium its characteristic "humped back" shape.

Laterally, we have the alar membrane for the wing attachment above and the episternum and episeron below. The mesosternum is large and well marked. The mesothorax bears the halteres and the metathoracic spiracle or its plural aspect.

WINGS.

The two wings are borne at the sides of the scutum of the mesothorax. They are attached by means of the scleretes at the base of the wings to the alar membrane.

They are broad and characterized by having only the veins of the anterior portion of the wing developed, the other veins showing only as thickened , but transparent tracings. Following the nomenclature of Johannsen for this family, I describe the venation of the wing as follows: the anterior edge of the wing is reenforced by the costa which extends to a point some little distance before the tip of the wing. The sub costa joins the costa at a point about one-third the distance from the base of the wing to the tip. Radius is all but fused with subcosta showing up as a clear line along the posterior edge of subcosta for 3/5 of its length - (Subcosta), it then appears as a separate vein. R. joins costa a little beyond the point where subcosta meets costa. This point is marked by a slight emargination in the costal margin of the wing. $R_{2+3+4+5}$ extends for a considerable distance, finally meeting costa shortly before its termination-(Costa) The base of media is well marked. This well marked portion extends for the first 1/6 of the length of the vein. There a cross vein connects media with Radia and the vein becomes transparent soon branching into M_1 and M_2, M_1 extending to the tip of the wing and M_2 running out along the posterior side of the wing. Cubitus is represented more as a fold than as a vein. There are two clearly marked anal veins, Anal 1 being straight, and anal 2 curved, The anal area lying posteriorly to the anal veins is creased by two folds.

Legs.

The legs of Simulium are stouter than those of

most of the related forms. The following segments are present: Coxae- a strong cylindrical segment - trochanter narrow and somewhat irregular - Femur rather stout, Tibia about the length of Femur, also stout, Tarsi, joint one about as long as the remainder joints taken together. Five joints and Tarsi - the last bearing simple claws.

ALIMENTARY CANAL.

The alimentary canal is a comparatively straight tube extending from the buccal cavity, which lies at the base of the clypus, to the anal opening in the last segment.

The Proboscis.

This is made up by the close application of the hypohharnyx to the labrum and ensheathed by the fleshy labium. The tube thus formed leads up to the buccal cavity which is slightly, if any enlarged. The buccal cavity leads to the pharnyx which is so constructed and controlled by muscular attachment that it serves as a pumping apparatus, by means of which the blood is drawn up between the labrum and the hypopharynx into the mouth.

The Oesophagus.

The oesophagus joins the pharynx at right angles to it, this union being branded by a strongly chininized collar, the upper extremity of the pharynx. It is smaller than the pharyngial box and is flanked on the dorsal side for a short distance by two chininized plates. This portion lies between the sub and supra oesophygal ganglia. There arises from the dorsal wall of the oesophagus, just

caudad of the chitinized portion, a muscle that extends upward and backward to take its insertion in the wall of the vertex of the head. Plate IV, fig. II. The oesophagus narrows as it passes from the head to the thorax, being at this point little larger than the nerve chord just below it. (see fig.1) Plate IV) Upon reaching the thorax it may change its direction slightly and continue its way thru the thorax to the abdomen or it may make a sudden bend dorsally and as abruptly bend back again, then continueing its course from there on as in the other case. In any event the slender portion of the tube suddenly enlarges forming a tube of three times its diameter, The union of these two parts being marked by an enlargement or a series of three enlargements. From the ventral side of this enlargment there arises a thin walled duct that leads backward beneath the canal and expands into a rather large shapeless pouch. This duct cannot strictly be defined as such for it is simply the cephalic end of a sac which gradually narrows till it joins the digestive canal at the point shown in the figure (Pl IV, fig. I) The walls of this pouch are extremely thin and transparent and made up of a single layer of cells, in stained mounts appearing as in Fig. Pl. This sac by virtue of its position and union with the proventriculus leads to the conclusion that it must function as a food resevoir. Its general position is similar to that of the sucking stomach of Stomoxys Calcitrans figured by Brain '12. But the failure to find a well defined muscular coat makes it probable that it is solely for storing food.

The Stomach.

The digestive tract upon passing into the abdomen usually turns dorsally for a short distance where it enlarges to form an oval shaped organ, the ventriculus or stomach. No coecal tubes have been found, but the sufface of this mid-intestine is irregular and it may be that the glandular areas in the wall of this organ will prove to function as caecal glands.

Malpighian Tubes.

Immediately below the mid gut there arise two tubes which directly branch into two. These four tubes are of great length and lie folded and doubled back on themselves above the mid intestine and above and around the hindgut. These are the Malpighian tubules. They are made up of elongated secretory cells arranged as shown in Fig. Plate VII. They are best studied in the male and freshly emerged female. Vaney '02 finds that these tubes pass from the larva to the adult without any transformation, so that an examination of the larva gives a fair idea of their position in the adult. Just caudad of the region where the malpighian tubules are attached the hind gut takes a turn dorsally to a point below the junction of the fifth and sixth segments and then bends caudad at right angles to about the seventh segment where the tube becomes first constricted and then dilated, especially on the lower side, which gives the appearance shown in fig. II, Plate II. This rectal enlargment is a sac, the wall of which is distinctly striated , see Fig. Pl.

and contains suspended from its upper or cephalic end six cone shaped pappila or glands; The rectal glands. Each of these is made up of glandular cells containing large nuclii. These cells are shown in cross section to be six in number Fig. Pl VII and longitudinal sections show that that there are layers of these cells. Each papilla is surrounded by a transparent membrane shown in fig. pl VII The fact that they are characteristically glandular and lack the tracheal branches figured by Miall and Hammond for Chironomous fly, lead to the conclusion that they do not function as respiratory organs as Leydig has inferred is the case with some insects.

Salivary Glands.

Beside the Malpighian tubules and the food resevoir there are two other organs leading into the alimentary canal. Both of these join the tract before it leaves the head. The first is a pair of organs connected by a common duct to the base of the hypopharynx. See Fig. II Pl. IV, and the second, a long slender gland joining the canal at the point shown in Fig. I Pl.IV The paired organs are the salivary glands which lie in the fore part of the thorax on either side of, but dorsally to the oesophagus. They are connected to the common duct beneath the aesophagus by means of slender ducts see Fig. II Pl. IV. They lie so close to the prothoracic wall and so near to the large tracheal trunks of the meso thoracic spicacle that they are often difficult to demonstrate. They lie on either side, between the second oblique and first longitudinal layer of muscles. Structurally they are in two parts. There is any upper

part which when stained shows itself to be glandular see fig pl and a non glandular part ar sac. The glandular part is shown in sections to consist of thick walled pouches, the cells of which contain large nuclii. In structure they resemble somewhat the acinous glands figured by Packard (after list) . They do not at all resemble the trilobed glands of the mosquito or the slender glands of stomoxys. They appear to be somewhat similar to those figured by Newstead for Phlebotmus. The single organ lies above the proventriculus for the entire length of the thorax, enlarging slightly and terminating in the extreme front of the abdomen. Its duct has been traced down over the left side of the proventriculus into the head.

THE RESPIRATORY SYSTEM.

The respiratory system consists of two longitudinal trunks running from the head to the posterior end of the abdomen. These are connected in the thorax by a large commissure, and in the head by a small one. The head and thorax are well supplied with large tracheal branches while the abdomen possessed a great net work of tiny threadlike tubes arising from the rather small tracheal trunks. These little tubes ramify all parts of the abdomen, binding together the loosely joined ova of the female, interlacing the many loops and folds of the malpighian tubes and tying the fat body with all to the digestive canal. Thus we find in the abdomen on either side a longitudinal tracheal trunk, and throughout, a mat of slender tracheal filaments.

These tracheal trunks are not straight tubes, but curved at the six points where the branches leading to the abdominal spiracles are given off, namely at the points between the second and third, third and fourth, fourth and fifth, in the fifth and in the sixth segments. The spiracular openings being located on the lateral surface of the body as follows:- 1st one in the 2d segment near the junction of 2d and 3d, 2d one in 3d near the junction of 2d and 3d, 3d one in middle of segment 4, 4th one near the middle of segment 5, 5th one near the middle of segment 6, 6th one in 7th segment near its union with segment 6. These spiracles show up indistinctly as dark dots on the gray surface of the insect. They are not functional in the larva and pupa, their work being taken over by the blood gills in the larva and by the cuticular gills in the pupa.

Taylor '02 finds that the general scheme of tracheation is however about the same in the larva and pupa stages as in the adult save for the method by which the air is brought into the system mentioned above. His studies were upon Simulium latipes and in his researches he records but five abdominal spiracular branches, failing to find the one in the second segment which he thinks however must exist in view of the fact that the cast pupal skin shows an attached remnant of such a branch. He also inferrs that one exists in the first in some vestiginal form but I am unable to confirm this.

In the thorax there is a tendency of the tracheal branches to be dilated, and the fact that there are several large trunks or branches originating at the metathoracic spiracle and extending forward in different planes of the thorax makes it difficult to distinguish the main trunk from the others. In fact if my interpretation is not incorrect, the main trunk is much smaller than the very large branches that are sent off, see fig I Pl.III This main trunk extends from the metathoracic spiracle underneath the large oblique thoracic muscles and runs along dorso laterally to the fore gut, turning up before the first oblique muscle to meet the other branches extending from the mesothoracic spiracle. It then continues to the head where it breaks into many branches. These main trunks are connected in the thorax by one prominant commissure arising from the caudal end of the anterior third and looping up over the alimentary canal to the top of the second longitudinal muscles and thense back in the same plane between the right and left longitudinal thoracic muscles, joins the corresponding trunk on the other side. From the metathoracic spiracle there arise the following branches one extending dorso-cepholed, laterally to the main muscles of the thorax for some distance, then narrowing suddenly, it turns in under or between the outer oblique muscles, four and five and gives off one small branch, another has the same general direction but lies in the plane between 2d oblique and first longitudinal and extends to the thoracic

wall where it turns ventro-cephalid at right angles and drops beneath the sixth longitudinal muscle. Another in this same plane extends ventrally supplying the legs, while another leads forward to join the system again at the mesothoracic spiracle.

At the meso thoracic spiracle we find a number of branches, the direction of which may be best observed by referring to the drawings.

The mesothoracic spiracle lies well toward the front of the thoracic box and might well be mistaken for prothoracic in origin, but Taylor gives the following reasons for considering it meso thoracic - "Immediately in front of the anterior thoracic spiracle of the fly a well marked apodeme or thickening of the cuticle runs obliquely backward to the mid-ventral line, where it unites with a similar thickening on the other side. The fore leg of the fly is inserted on the body in front of this apodeme, and muscles from the leg are attached to it. As the apodeme approaches the mid-ventral line, it bears the ante-furca, which, wherever it occurs, marks the junction of the pro and meso thorax. The anterior spiracle of the thorax lies close behind the opodeme which carries the antifurca, and is therefore mesothoracic in position.

SEXUAL ORGANS OF THE FEMALE.

The female Simulium like most other related forms possesses two ovaries which lie on either side of the alimentary canal. These may occupy only the caudal third of the abdomen in case of the freshly emerged female or take up all the available space in the abdominal cavity even crowding into the thorax abound the foregut in case of females ready to oviposit.

When the newly emerged female is examined, the ovary appears as a transparent pouch showing practically no differentiation. But when this is removed and stained with Borax Carmine the ova are deeply stained and are seen to lie in regular order within the ovarian sac - see fig Pl.V As the ova mature they lose their regularity of order and individual shape, so that, by the time they mature they are but loosely joined together and extremely irregular in outline. The latter is brought about by their crowded condition in the body of the insect. The eggs at maturity are ____ mm in diameter and when we are able to count as many as 276 ova of this size packed in the body of a single female we do not wonder that they are so angular in form.

The oviduct connecting the ovarian sac with the exterior is comparatively easy to trace in the early adult stage, Here the oviducts are seen to join forming a short tube "the vagina" which passes to the genicular opening on the ventral side of the body between the penultimate and antipenultimate segments. As the eggs pass down the common oviduct they are fertilized by sperm which has been

stored since the mating with the male, in one small chininized spherical pouch, the sperm athecae- see fig II pl V This spermatheca is ___ mm in diameter and dark brown in color with a thick chininous covering. It is attached to the distal end of the common oviduct by a white cylindrical tube. The attachment of this white tube to this hard brown sphere reminds me of a germinating seed. It is interesting to note that with this fly there is but one functional sperm-athea. In all the related species of flies that have been studied there are two - Phlebotomus, Musca domestica. Scalcitians, Culex and the Cheronomids have two.

Since in primitive insects the oviducts were separate, opening to the exterior thru separate orifices we would expect that whatever evaginated pouch might have been formed for the storing of sperm from each of these tubes would persist even after the coalescing of the distil ends of the oviducts. In other words we would expect a pair of spermatheca if such existed before the union of the two ducts. The finding of hit one must be explained as a specialization in advance of Chironomus, for instance which is figured by Miall and Hammond as possessing two.

SUMMARY.

This paper in its complete form is to be the first of a series of three papers, the others dealing with the Histology and Embryology of Simulium flies.

In our study of the gross structures of the sand fly, we find the following:-

The mouth parts are fitted for rasping and represent only one of a few cases in diptera where all of the mouth parts are present.

The food is taken into the hiceal cavity by a sucking apparatus on the head which is controlled by muscles.

The digestive tract is fitted for digesting liquid food.

The food is acted upon by the secretion of a pair of salivary glands and probably also by a secretion from an unpaired gland located above the fore gut. There is a food resevoir which serves as a temporary storing place for the food. No coecal tubes have been found.

There are four long malpighion tabules and the mid intestine is made up of large elongated cells.

The digestive canal terminates in a large rectal pouch which contains six rectal papillae. These are glandular and not connected in any way with respiration.

Taking the study as a whole we find the systems enough different from closely related forms to make its persuance intensely interesting and profitable.

The structure of the digestive canal with its glandular appendages makes it possible that protozoan parasites of some kind or another find a home for part of their existance at least in these insects.

BIBLIOGRAPHY.

1844 - Anatomie Generale des diptera apparcil digestif,
Ann Sc. Not. Sir. 3 Zool 1-1844 p248-372

Trophi Depterorum and de en cephale Myggilarvor.

1870 - Tranaformation of S. Osten Sachen - Am. Ent. Vol 2 p 229

1874 - On the Structure of Mouths of Insects Sci. Goss. p 107
Distribution and primitive number of Sperocles in insects - Packard Jr. A.S. Am. Not VIII p531

1879 - Recherches morphologiques et zoologiques sur le system nerveux des insects dipteres.
Kunchel d'Herculais -C.R.IXXXIX
p 491-94

1880 Sur la construction des organs hiccaux chez les diptera Meinert F. Eit Fidshr 1 p 150-153

1881 - Anatomy of mouth parts and sucking apparatus of diptera. - Dinnoch G. Psychi iii 4-60 p 231

1885 - Head and Mouth of Insects -Barthelemy (18)
Halteres of Diptera - Sec. (185) French.
Optic ganglion of Diptera - Viallanes (346-347) Fr.

1886 - Instructions for students for preparation of insects for dissections P 31-42 De Witz "Anlerting zur anfertigung und aufbewarhrung. zootmoischer Praporate"

1886 - Various papers on nervous system.
Zool Rec. p77
Summary of Viallanes paper '85 in optic ganglion of some dipterous larvae. J.R.Mier Soc. (2) VI p 430
Abstract of Kraepelin's paper, (1883) on organ of smell in Artheopods Am. Not. XX p889-94-973

1887 General Anatomy of larva of Culex memorosus Raschki - (600-601) Zool. Aug. X p 18-19

Structure of the head of a blow fly.
Lorme B.T.J. Quek Club. iii p 120-124
Salivary glands - Knuppel (378)
Malpighian vessels Chotodkovsky (130)

1888 - External Anatomy of Muscides -Pandelle (699)
French - Rev. d'Ent. VII 258-362

1889 Anatomy of Insects Lowne (562)
J.Quek Club. iii 373-386

1890 - Anatomy of Culex in pupal stage Herst (390)
Strad. Dec. Call ii p 47-71

1890 - Mouth parts of Diptera -Smith J.B.
Fr. Am. Ent. Soc. XViii p 319
Anatomy of balancers of diptera
Weinland (899)

1891 - General anatomy of Culex sp Hurst (430)
Fran March Micro Soc 1890
Brief neg. Statement as to muscles of halteres of Diptera - Lendenfeld - Zool. Aug. XIV p 63

1893 - General Anatomy of larva and pupa of Dicromora
Miall (634) Fran ent. Soc. London.
1893 - p 235-253
Mouth organs of Diptera
Ann. Not. Hist. (6) XI, p 45-46
Maplighean Tubes, their primitive number homologies - Wheeler (1050)
Psyche VI p 457-561

1895 - Blood sucking Diptera Townsend (1142)
Jr. N.Y. Ent. Soc. iii P 134

1896 - Morphology of mouth parts Smith
N. Am. Phil. Soc. XIX p 175-198

1897 - Anatomy of Diptera
(Sections of tarsus, head, sperocles
Jenkennson p 173- 183
Int. J. Mier VII

1897 - Trophi, muscular apparatus of oesophaus of Tobanus Steinheil - (1060)

Structure and life history of Pholacrocera replicara with append. of lit of earlier stages of the cylindrotomina (Diptera)
Fr. Ent. Soc. London. 1897 p 343

1898 - Alimentary canal of Drosophiaa.
Berlese (77)

1900 - Anatomy and life history of the Culicidae.
Handbook.
Giles G. M. London. 1900 -- 374
Philiniern of diptera Wesche (1406)
Comparative anatomy and homologies of the mouth parts of diptera Kellogg (626)
Psyche VIII p 303, 327, 346, 355, 363.

1901- External structure of culicidae, imago, pupa larva, egg, Theabold, Royal Soc . London, 19o1 8/10
Vol. I XVIII 424 pp

Anatomy and Histology of adult female mosquito
Royal Soc. London Rep. Malaria Committee IV 20 pp

Structure of Typhoid larva Kellogg (713)

1901 - Ovaries and oogenesis in Culex and Anophiles -
Kulargein (795) German

1902 * Anatomy of Dolichopoda Monei (Ranan)
Tracheal System of Simulium, larva,
pupa imago - Taylor Fr. Ent. Soc. London 1902
701-716
Male Gentilia of Asilidae - Snidgross
Psych. IX p 399
Mandibles of Diptera - Longhoffer

1903 - Structure of abdomen diptera Barner
German - zool Aug. XXVI p 495-508
Alimentary system of aupphales
Nuttall and Shipley.
J. Hygiene iii p 166-215 find!

1904 - Comparative anatomy of gentilia-
Tipulidae - Snodgrass.
Am. Ent. Soc. XXX p 179-236.
Morphology of head of dipterous larvae and
shaped sides of oesophagus of Musca.
Holingien (German) Zool Aug. XXVII pp 343 .
1904 - Trophi of Diptera - Wesche'
The larial and maxillary palpi in Diptera
Fr. Latin Soc. London IX p 219-230

Mouth parts of flies - Winner (Russian)
Structure of proboscis Anophilides Leon (German)
Anatomy of mosquito, librido
Revist. Medicina Trop Apr. 1904
Structure of Conopidae and larvae De. Meijere
Tydschi Eat. XIVI p144
Zur. Morphologie des inseklen kopfes. Holmgren.
Zeitschi wiss Zool IXXVI p439
Zur Morphologides des insektenhopfes einiges uber
die Reduktion des kopfes der deptereh larvan. Zool.
Aug. XVIII p 343

Zur. Vergluchenden Morphologie und systematekder
Embuden. Zugleich 8
Betrag zur. Kenntness of Thoraxder.
Acta - ac - German IXXXII nO. 2p 65
Notes on Structure of thorax and maxilla of insects
Bands Pent. Soc. Wash. VI. P 149-153.

1905 - The Genitalia of the tsetsi fly J. Quckitt Club.
p 233-238

1905 - Welschi W. The mouth parts of the Nemoceia and
their relation to other families of Diptera.
J. R. Miero Soc. 1904 p 28-107

Labial and Maxillary palpi in Diptera.
Fr. Linn Soc. London OX p 219
Morphologie der Embiiden - Adeluarg N.
Zool. Zentralbl. 580-585
Die Tsetsi Fliege (Analing of Glossina)
Sander L.
Leipzig 8 vol. 79 pp
Report on the anatomy of Tsetsi fly Minchen E.A.
P. R. Soc. London IXXVI p 8-31
Structure and life history of Psychords sex punctate
Fr. Ent. Soc. London p 293 by Dell J.A.

1906 - Headlee - Bloodgills of Similium pictipes.
Am. Nat. 40- 1906 - 875-885)
Internal Anatomy of Stmoxys Tullock.
London Proc. R. Soc. 77 B 1906
p 523-531

1907 - The genetalia of both sexes in diptera and their relation to the armature of the mouth.
London - Trans Linn Soc.
Antennae of Diptera Williston.
Biol. Bull. Woods Hall,Mass.
13 -324 1907.
Internal structure of insect heads - Hatt W.
Manchester Trans Miero Lei 1906
84-86

1908 - Anatomy of horse fly Hewitt C. G.
Quart. J. Miero Sci. London 51- 1907
p 395
The proboscis of the blow fly Wesche W.
London J. Quek miero cl. ser. 2, 10-1908
p 283-294

1909 An illustrated glossary of chaetotoxy and terms used in describing Diptera.
Ento News Phil, Pa 20, 1909 p317
The anatomy of cheronomus pusio - Mundy A. T.
Leicester 1909 4-56
Anatomy of larva of Musca dermestica.
Hewitt C. G.
I. J. Microsi Sci. London 52, 1909
p 495-543

1910 - Les Insectes (Anatomil et physiolge generales
Houlbert Paris (DOm Ency. P 361-
Some points in the anatomy of the larva of Tipula maximo - London - Tr. Linn. Soc. 11- 1910
c 125 - 1353
J. M. Brown.
Structure and metamorphosis of the alimentary canal of the larva of Psychoda alternata.
Haseman, Leonard, Ann. Ent. Soc
Am. Columbus, O

1912 - Stomoxys Calcetrans - Brain C. K.
Ann. Ent. Soc. Of Am Dec. 1912/

1911 - Papataci Flies (Phlebotomus) of the Maltese Islands - Bull. of Ent. Research - May, 1911.

General Texts.

Packards - Text Book of Entomology
Folsam's - Entomology
Miall & Hammond - ~~Horligriun~~ Fly.
Henneguy's - Les Insectes
Willestin's - Diptera
Johannson's - Aquatic Insects.

PLATE I

SIMULIUM VITTATUM (FEMALE)

Ms- meso thoracic spiracle.

am - alar membrane

mn - mesonatum.

In the wing: c - costa

R- radius I

R_{2-3}etc - rest of radial branches.

M- median

cu - cubitus.

a - anal

f - fold in wing.

PLATE II

Figure one.

Fore portion of fly:

The two left oblique layers of muscle, and the left longitudinal removed.

Caudal portion of fly:

The ovaries of a recently emerged female.

Sg - salivary gland (left)

d - duct of salivary gland.

a - tracheal commissure.

M - newve cord

Pr - Proventriculies.

G - solitary gland.

ov.- ovary

sp - spermathea.

Figure Two.

Digestive canal.

st. - stomach.

m - malpighian tubules.

F.R. - food resevoir

r.g. - rectal gland.

r.g. - A,B and C terminal and lateral views rectal pouch.

s.p. spermathea.

PLATE III

Figure I

Left oblique muscles removed.

m - muscle (left Longintudinal)

a - integument

b - metathoracic spiracle

n - nerve ganglia

c - s. gland.

Figure II

Surface view of trachial system -

m - mesothoracic spiracle.

me - metathoracic spiracle.

PLATE IV

Figure one .

A. Head parts dissected - the digestive system shown to the cephalic end of abdomen.

B. Chitinized plates on oesophagus.

l - labrum

m - mandible

mx - maxilla

S.d. salivary gland.

P - pharynx

Pi - Pharynigial ring.

Mu - muscle.

L - gland

Pro.- Proventriculus

f.r. Food resevoir.

Figure Two.

Head dissection study.

s.d. - salivary duct.

M - muscle

oe- oesophagus

S.g - salivary gland.

ovi - duct to food resevoir.

PLATE V

Figure 1

Sectional view of female genitalia.

ov - ovary

ovi - oviduct

sp - spermathrea

r.p. - rectal pouch

Figure II

ov. - ovary

ovi - oviduct

a - a chitinized rod to which muscles governing the genitalia are attached.

Figure III

Female genitalia as seen candad.

a - chitinized rod shown in fig. 2 at a.

b - anus.

c - genicular opening.

Figure IV

above shown laterally.

PLATE VI.

Figure II

Musculature of Head.

Figure I

Salivary glands.

PLATE VII.

Figure 1. Portion of Malpighian tubule.

(a) Nucleus.

(b) Cell.

Figure 2. Cross section of Malpighian tubule.

Figure 3. Longitudinal section of Rectal pappila.

Figure 4. Cross section of the R. P.

PLATE VIII.

Figure 1. A reconstruction of the head of the female fly.

(a) brain.

(b) Ventral cord.

(c) Buccal cavity.

(d) Muscles controlling the pharynx.

(e) Portion of tentorium.

Figure 2. The mouth parts with their muscular attachments.

Plate IX.

Drawing of the longitudinal section of the female fly slightly reconstructed.

(a) The brain.

(b) The ventral nerve cord.

(c) The muscles of the thorax.

(d) The Oesophagus.

(e) The valve at the junction of the Food resovoir and Proventriculus.

(f) Food resovoir.

(g) Stomach.

(H) Malpighian tube.

(i) Ova.